Supplementary Material

Severe Gestational Low-Protein Intake Impacts Hippocampal Cellularity, Tau, and Amyloid-β Levels, and Memory Performance in Male Adult Offspring: An Alzheimer-Simile Disease Model?

Supplementary Methods

MWM proximity measure

The proximity measure optimized computer tracking to identify a rat's position relative to the target location; it is considered highly sensitive to age-related learning/memory impairment in aged rats [1]. This study uses new proximity measures to the behavioral analysis of learning in the water maze task. We did this to test the memory/learning ability based on the proximity measure for use in characterizing individual differences in the effects of aging on spatial learning.

The need for such an index was based on the previously identified limitations of the customary analysis and on certain special features of the effects of aging on spatial learning ability.

The present study used the proximity of the animal's position to provide several training trials and probe trial performance analyses. The proximity measure was obtained by sampling the animal's position in the maze (10 times per second) to record its distance from the escape platform in 1-s averages.

By this method, scores obtained with the proximity measure are designed to reflect search error; they represent deviations from an optimal search, from a direct path to the goal (hidden platform) in the water maze.

The assessment of proximity to the target provides a more efficient analysis method than the multiple measures traditionally used to characterize probe trial performance: platform crossings and path length or time in the quadrant.

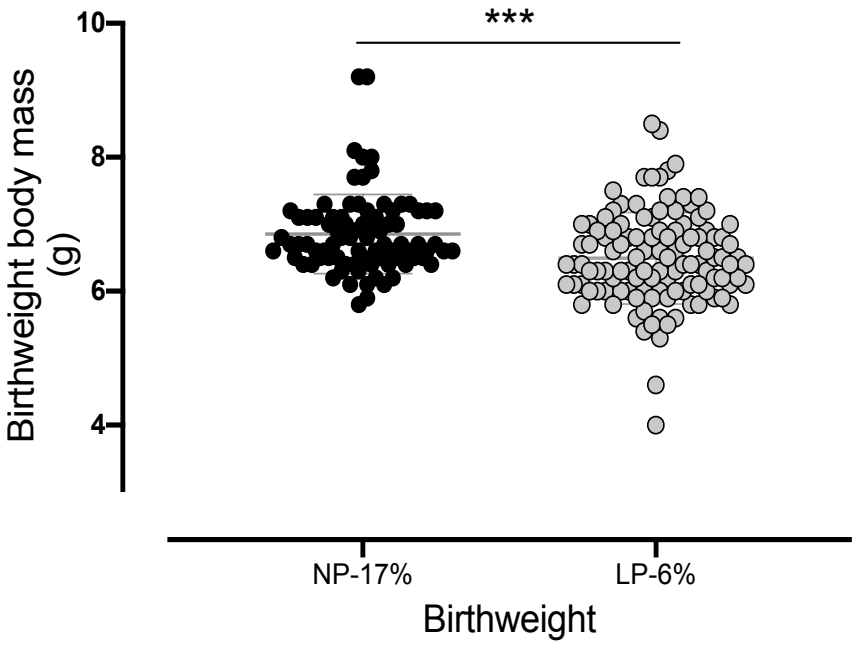
An analysis of young rat performance that used this measure showed rapid acquisition of improved search accuracy during the interpolated probe trials. Comparison of young and aged groups demonstrated that age was most pronounced relatively early in training because aged rats acquired a spatial bias more slowly. Thus, in this study, the probe trial analysis using proximity to the target was sensitive to an age-related impairment in spatial learning.

It appears that some rats become less proficient in learning the information that is required for efficient navigation to a specific location. So, the method used here offered a sensitive, efficient, and valid approach to assessing this age-related cognitive deficit.

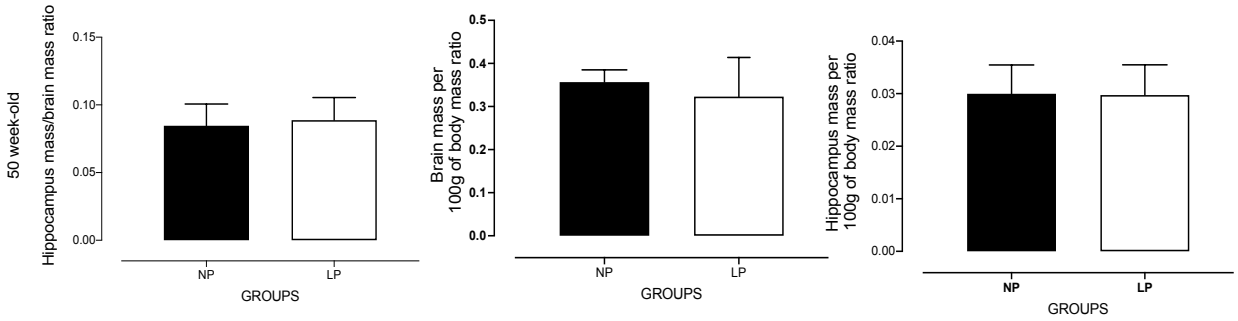
REFERENCE

[1] Gallagher M, Burwell RD, Burchinal M (2015) Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris Water Maze. *Behav Neurosci* **129**, 540–548.

Supplementary Results



Supplementary Figure 1. Offspring bodyweight (g) at birth NP progeny as compared to LP offspring. Results are depicted as scatter dot-plot and are expressed as means ± SEM; the comparisons involving only two means within or between groups were performed using a Student's *t*-test. Welch's *t*-test was used to correct situations characterized by heteroscedasticity. When statistically significant differences were indicated between selected means, posthoc comparisons were performed using Bonferroni's contrast test. The level of significance was set at $*p < 0.05$.



Supplementary Figure 2. Offspring brain and hippocampal masses (g) in 50-week-old NP (n=5) compared to age-matched LP (n=5) offspring. Results are expressed as means ± SEM; comparisons involving only two means within or between groups were performed using a Student's *t*-test. The level of significance was set at *p < 0.05.